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Adrenal Hormone Responses to Cerebroventricular Angiotensin II in the Sheep (41565)

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Abstract. The effects of intracerebroventricular angiotensin II on plasma aldosterone levels were studied in conscious and anesthetized sheep. Infusion of angiotensin II at 20 ng/min into the lateral cerebral ventricle raised plasma aldosterone levels. Since plasma cortisol increased in parallel with aldosterone, and as the response of both corticosteroids was obliterated by dexamethasone pretreatment, ACTH appeared to mediate the adrenal-stimulating action of angiotensin II. Central infusion of angiotensin II, was without effect on the peripheral renin–angiotensin system or the sympathetic nervous system as gauged by the lack of change in plasma levels of angiotensin II, norepinephrine, and epinephrine.

There is considerable evidence that the cerebral renin–angiotensin system functions as a regulator of water and electrolyte homeostasis. Much interest has been focused on the effects of angiotensin II (AII) or blockers of the renin–angiotensin system, administered into the cerebroventricular system, on thirst (1) and ADH release (2, 3). In contrast, less attention has been directed toward possible interactions of the cerebral renin–angiotensin system and the most potent adrenal mineralocorticoid, aldosterone. Since intraventricular (IVT) administration of AII has been reported to alter plasma levels of the aldosterone-secretagogues ACTH (4–6) and renin (7–9), physiologically important relationships between the cerebral renin–angiotensin system and aldosterone secretion seem possible. The present experiments in sheep were planned to complement recent data from dogs (10) which indicated a brain–adrenal interaction.

Methods. Intraventricular infusion studies were performed in healthy 2- to 3-year-old Corriedale whethers, weighing 36–51 kg. In all, 15 experiments were carried out in 12 animals, 3 being used twice with a 1-week interval between experiments. One such "pairing" was present in each group. There were three study groups: the first, acting as controls (N = 4, 2 anesthetized and 2 conscious) received an artificial cerebrospinal fluid (CSF) solution throughout; 2 of these received the infusate containing AII delivered into a cerebral sulcus, because of a misplacement of the needle. A second group was infused with AII (N = 7, 2 anesthetized and 5 conscious). The third group received AII after intramuscular dexamethasone administration in order to inhibit adrenocorticotrophin (ACTH) secretion (N = 4, 2 anesthetized and 2 conscious).

The sheep were held in pens with access to commercial sheep pellets, oat chaff, meadow hay, and cabbage for 2–9 days prior to surgery. After fasting overnight, the animals were anesthetized with sodium pentobarbital (30 mg/kg, iv); maintenance boluses of the barbiturate being administered thereafter as required. In one sheep, anesthesia was maintained with a nitrous oxide–oxygen mixture. The sheep's head was held rigid in a stereotaxic apparatus and a 22-gauge needle, 37.5 mm long, was advanced under sterile conditions into the right lateral cerebral ventricle. Entrance into the ventricle was signaled, as previously described (7) by a sudden fall in pressure. Pressure was monitored by a transducer connected to the needle during continuous infusion of an artificial CSF solution at 10 μl/min. After successful placement of the needle and its fixation to the skull with dental arcylic and metal screws, a "control" infusion of artificial CSF at 40 μl/min was initiated and continued for 70 min. Venous samples were drawn after 60 and 70 min (control sam-

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ples). The IVT infusion was then changed to angiotensin (Hypertensin, Ciba, 500 ng/ml) in the artificial CSF, and the rate of administration continued at 40 μl/min. Venous samples were again obtained at 60 and 70 min of the infusion period (experimental samples). Finally, artificial CSF alone was infused, again at 40 μl/min for a further 70 min, and two final venous samples were drawn at 60 and 70 min (recovery samples).

Nine sheep were permitted to regain consciousness, and were studied unanesthetized, 3–9 days later. For these conscious experiments the animals were again fasted overnight and held in a restraining cage for at least 12 hr prior to study. The experiments were carried out as described above, with sequential control, experimental and recovery phases and venous sampling at 60 and 70 min of each phase. Since results from anesthetized and unanesthetized animals were similar, all data were pooled for presentation.

Four sheep (two anesthetized and two unanesthetized) were given intramuscular dexamethasone, 4 mg, 2–4 hr prior to the IVT infusion of AII, in order to determine whether or not AII-induced hormone changes were modified by suppression of ACTH release.

Upon completion of the studies, blue dye was injected intraventricularly to verify needle position. When staining of the ventricular system was seen at necropsy, needle siting was considered satisfactory. In two animals the needle was found at necropsy to be sited in a cerebral sulcus so that the infusate containing AII could not have reached the ventricular system. Data from these animals were included in the control group of animals. To complete this control group, two sheep received IVT infusions of artificial CSF, without the addition of AII, and venous samples were withdrawn at 60- and 70-min intervals of the control, experimental, and recovery periods: accurate placement of the needle in the lateral cerebral ventricle was confirmed at necropsy.

Venous blood was drawn through an indwelling jugular catheter into cooled containers, centrifuged immediately at 4°C, and the plasma stored at −20°C (or 4°C for electrolytes) until analyzed. A total of 30 ml of blood was sufficient for each set of analyses. Established methods were used to measure plasma cortisol (radioimmunoassay Premix kit, Diagnostic Products Corp.), aldosterone (11), AII (12), and catecholamines (13). Plasma sodium and potassium levels were measured by flame photometry. All samples from a single experiment were analyzed in a single assay run.

Results are presented as the mean ± standard error of the mean. Statistical analyses were carried out using the *t* test for unpaired data when comparing results from separate experimental groups, and Student's paired *t* test for data within the same group of animals.

Results. Hormone and electrolyte data from the three experimental groups are shown in Table I, the results within each group being categorized as control (C), experimental (E), and recovery (R). AII levels were low and showed no pattern of response in any of the experimental groups. Nor was there any effect on plasma levels of epinephrine or norepinephrine by AII. Na and K levels were also unaffected.

Figure 1 presents the data for aldosterone. In the time control series, no significant change in plasma aldosterone levels were noted throughout the experiment. However, on infusion of AII, aldosterone levels rose significantly, and returned toward control levels during the recovery period. In contrast to these results, if the sheep were pretreated with dexamethesone before beginning the experiment, AII infusion had no effect. The decline in aldosterone levels was not significant. No significant difference was found between the control levels of aldosterone for the three groups.

Cortisol levels also increased on AII infusion from 168 ± 46 n*M*/liter to 195 ± 87 n*M*/liter ($P = 0.03$, one tail), and returned to control levels during the recovery period (166 ± 46 n*M*/liter). Values for the time control were unchanged for the three periods and were: 210 ± 55, 182 ± 39, and 193 ± 44 n*M*/liter. Although the mean cortisol level in the control animals was higher than in the group which received AII, that difference was not statistically significant. Cortisol levels in the dexamethasone treated sheep were too low to be measured.

Discussion. The peripheral renin–angiotensin system plays an important role in water and electrolyte homeostasis, in part by its action to stimulate release of aldosterone from the adrenal glomerulosa. There is now considerable evidence suggesting that the cerebral

TABLE I. HORMONE AND ELECTROLYTE DATA

Plasma concentration		Control (N = 4)			Angiotensin II (N = 4)			Dexamethasone + AII (N = 4)		
		C	E	R	C	E	R	C	E	R
Angiotensin II (pg/ml)	Mean	17	22	22	21	23	25	22	22	24
	SE	2	4	3	7	6	7	4	6	8
Epinephrine (nM/liter)	Mean	0.62	0.52	0.52	0.44	0.45	0.55	0.45	0.44	0.52
	SE	0.19	0.11	0.13	0.11	0.08	0.14	0.14	0.08	0.05
Norepinephrine (nM/liter)	Mean	2.35	3.07	2.93	1.86	1.93	2.16	2.42	2.53	2.87
	SE	0.28	1.14	0.95	0.30	0.17	0.36	0.60	0.76	0.66
K^+ (mM/liter)	Mean	3.92	3.80	3.85	3.66	3.72	3.66	3.30	3.17	3.16
	SE	0.17	0.11	0.11	0.15	0.16	0.18	0.27	0.23	0.12
Na^+ (mM/liter)	Mean	148	147	147	144	144	144	147	148	147
	SE	2	2	2	1	1	1	1	1	1

renin–angiotensin system likewise contributes to the regulation of water and electrolyte balance. Whereas the stimulatory effects of IVT AII on thirst (1) and ADH release (2, 3) are well documented, less information is available on the response of plasma aldosterone to central AII administration. The aims of the current studies in sheep were first, to document aldosterone levels during IVT AII infusion and second, to define what factor(s) mediated the aldosterone response.

Our results show that the IVT administration of AII induces a rise in plasma aldosterone. That the stimulatory effect was due to AII reaching the cerebral ventricular system, was confirmed by the lack of aldosterone response when the octapeptide was infused onto the surface of the brain (into a cerebral sulcus) or when the artificial CSF vehicle alone was administered into a lateral cerebral ventricle.

Which of the known aldosterone secretagogues might have mediated the stimulatory action of IVT AII? Plasma levels of AII, potassium, and sodium remained constant throughout the experiments and cannot be invoked. Our data suggest that ACTH, a potent short-term regulator of aldosterone secretion in sheep (14), was stimulated by IVT AII and induced a rise in plasma aldosterone. The pattern of change in cortisol concentration, which is accepted as an index of ACTH secretion, was parallel to that of plasma aldosterone. Further, dexamethasone pretreatment completely abolished the aldosterone response and presumably prevented any change in cortisol levels. These results are consistent with data from dogs (4, 6) and rats (5) which demonstrated AII to be a corticotrophin-releasing-factor. We now show in addition that the magnitude of the ACTH response to IVT AII is sufficient to release both aldosterone and cortisol from the adrenal in sheep.

Thus far we have assumed that the changes in plasma aldosterone reflect an increase in its secretion rate, but it might be argued that a decline in the metabolic clearance rate of aldosterone was contributory. Since any rise in circulating cortisol tends to increase, rather than decrease the plasma clearance of aldosterone, at least in man (14, 15), it is reason-

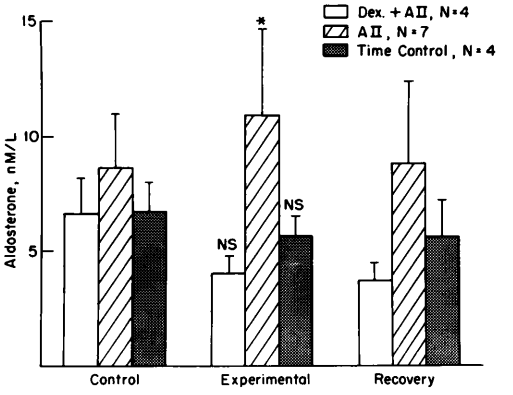

FIG. 1. Aldosterone levels of plasma following IVT infusions. Time control sheep received CSF infusion throughout the three periods. The other two groups received CSF only during the "Control" and "Recovery" periods, and AII during the "Experimental" period. *, $P < 0.05$ comparing experimental period to control period.

able to assume that the rise in plasma levels reflected an increase in aldosterone secretion. Indeed, since the metabolic clearance rate of aldosterone presumably increased as cortisol levels rose, the increment in plasma aldosterone may have underestimated the magnitude of the secretory response of the adrenal glomerulosa.

Whereas cortisol responses to IVT AII were similar in our sheep to those seen in the dog (4), the patterns of change in plasma aldosterone levels were opposite. AII infusion causes a decrease in aldosterone levels of plasma in the dog (4). We cannot with available data explain these discrepant findings. It is possible that aldosterone responsiveness to ACTH might be greater in the sheep than in the dog, either as a species difference or because the dietary intake of dogs prior to experimentation was low in sodium (15), or low in potassium (17) relative to that of sheep in the current studies. Dopaminergic regulation of aldosterone secretion is gaining acceptance (18), but what importance this system may have in different species and what modulating effect may result from IVT AII infusion remains to be seen. Finally, it is conceivable that an as yet unknown regulator of aldosterone (19, 20) was operative in the inhibitory effect of IVT AII observed in the dog, but not sheep, and was of sufficient potency to overcome stimulation of the zona glomerulosa by ACTH. Further studies are required to clarify this issue.

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